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1H-magnetic resonance spectroscopy in first episode and chronic schizophrenia patients

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Background/aim: The aim of this study was to compare metabolite levels of the dorsolateral prefrontal cortex (DLPFC), anterior cingulate gyrus (ACG), thalamus, and hippocampus in patients with chronic schizophrenia (CSPs) and first psychotic episode patients (FEPs) by the use of magnetic resonance spectroscopy (MRS).

Materials and methods: Thirty CSPs, 20 FEPs, and 30 healthy subjects participated in this study. N-Acetylaspartate (NAA), creatine, choline (Cho), and myoinositol levels of the DLPFC, ACG, thalamus, and hippocampus were measured by 1H-MRS.

Results: It was determined that the NAA/Cho ratio was lower in both the FEPs and CSPs than the healthy controls in the DLPFC. DLPFC Cho levels were also higher in CSPs than healthy controls. NAA levels in CSPs were significantly lower than in the control group in the hippocampus. There was no significant difference in neurometabolite levels and ratios in the ACG and thalamus between the groups.

Conclusion: This study supports neuronal dysfunction or loss of neuronal integrity in the DLPFC and hippocampus in CSPs. FEPs showed less neuronal dysfunction in the DLPFC, but not in the hippocampus. Our results suggest that schizophrenic patients show brain metabolic changes with the onset of the disorder in the DLPFC; these changes could be more apparent in the hippocampus as the disease progresses to chronic stages.

Key words: Schizophrenia, first episode, dorsolateral prefrontal cortex, anterior cingulate cortex, thalamus, hippocampus, magnetic resonance spectroscopy

1. Introduction

Schizophrenia is a chronic debilitating mental illness, which is characterized by thought, affective, and behavioral abnormalities and progresses with cognitive dysfunctions. Heterogeneity in clinical symptoms and the nature of neurocognitive dysfunction in schizophrenia suggests that several brain regions are involved in the pathophysiology of the disorder. Abnormalities in the prefrontal cortex (PFC), which is responsible for cognitive functions such as working memory, executive functions, and attention; the anterior cingulate cortex (ACC), which is responsible for integration of emotions and behaviors; the thalamus, which is responsible for interactions of cognitive and sensorial inputs in cortical areas; and hippocampal dysfunction and memory impairment are all known to have important roles in the etiology of schizophrenia (1).

Magnetic resonance spectroscopy (MRS) is an imaging technique that provides information about the neurochemical structure of the brain. The PFC and hippocampus are the most commonly investigated areas in schizophrenia by using MRS. The most commonly studied molecules are N-acetylaspartate (NAA), which is an important amino acid indicating the viability/integrity of neurons; choline (Cho), which provides information about cell membrane turn-over; creatinine (Cre), having a role in cellular energy metabolism; and myoinositol (myo-I), which is accepted as the marker for glial cells (2,3). Although it was reported that NAA decreases in the prefrontal lobe and medial temporal region including the hippocampus were a consistent finding in schizophrenia patients (4), no change in NAA levels could be found in the dorsolateral prefrontal cortex (DLPFC) of subjects with high genetic risk of schizophrenia (5), medicated patients with chronic deficit-syndrome schizophrenia (6), and medicated chronic patients (7) as compared to controls. However, several studies reported decreased PFC

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NAA levels in medicated chronic schizophrenia patients (CSPs) (8,9), first episode patients (FEPs) (7,10,11), and medicated schizophreniform spectrum patients (12). Similarly, several studies reported decreased hippocampal NAA levels (4), and a few studies reported unaltered NAA levels in chronic patients (13,14) and in medicated and unmedicated FEPs (15–17). Contrasting the above results, FEPs and drug-naive schizophrenia patients showed decreased hippocampal NAA/Cre ratios compared to medicated FEP (18).

The other most commonly investigated anatomic area in schizophrenia by using MRS is the anterior cingulate cortex (ACC), which is known to have strong reciprocal connections with limbic structures and to be an important contributor of the frontal cortex system (19). Several studies reported that there are statistically significant differences in metabolite levels in the ACC between CSPs and healthy controls (20–22). Lower NAA levels in schizophrenia patients receiving typical antipsychotics were reported when compared with the ones receiving atypical antipsychotics (23), and no difference in NAA levels was also reported in schizophrenia patients receiving atypical antipsychotics when compared with control subjects (24). When the literature is reviewed, studies about Cho, Cre, and myo-I metabolites in the ACC are limited. Many studies have supported that these metabolites do not differ in schizophrenia patients when compared with healthy controls (21,25,26).

In some studies that compared FEPs with CSPs, the PFC NAA/Cre levels were determined to be lower in CSPs when compared with healthy controls and FEPs (7,8,27). Başoğlu et al. compared 15 CSPs with 13 FEPs, and they reported that there were lower NAA/Cre ratios in CSPs and FEPs in the temporal cortex and thalamus when compared with healthy controls (28). Szulc et al. compared 31 FEPs with 17 CSPs and 13 healthy controls, and they reported that there was no significant difference in brain metabolite levels between the patient groups. They also reported that there was an increasing tendency in Cho levels in the temporal lobe in CSPs, a decreasing tendency in NAA levels in FEPs, and a statistically significant increase in Cho levels in the frontal lobe in both patient groups when compared with the controls (29). Along with differences between brain imaging techniques, there are limitations as some studies investigating FEPs and CSPs were focused on single brain areas such as the DLPFC, thalamus, or medial temporal cortex (8,9,29), and some of first episode studies included patients with psychotic disorders other than schizophrenia. Therefore, it is still unclear whether there are differences in neuronal integrity between FEPs and CSPs and which areas are related with the differences (15,18). Moreover, results regarding metabolites other than NAA are also inconclusive. While it was reported that Cho levels were decreased in the frontal cortex in some studies, they were reported to be increased in other studies, and there are few data related to myo-I levels indicating glial pathology (27,30,31).

In the present study, it was aimed to compare neurometabolite (NAA, Cho, Cre, and myo-I) levels in the areas playing a role in schizophrenia etiology such as the DLPFC, thalamus, hippocampus, and ACC in CSPs and FEPs who were clinically followed and confirmed diagnostically for schizophrenia with healthy controls. We think that our results may contribute to understanding whether schizophrenia shows any differences in neuronal and glial pathologies between the first episode and chronic patients, as well as knowledge about the possible degenerative or progressive nature of the disorder.

2. Materials and methods

In the present study, 30 CSPs and 24 FEPs were included. Inclusion criteria were age between 18 and 60 years old; fulfillment of DSM-IV diagnostic criteria for schizophrenia for CSPs; Clinical Global Impression score of moderate or above; illness history of longer than 3 years; and presence of one or more signs of delusion, hallucination, disorganized speech, disorganized or catatonic behavior symptoms, and short-term use of antipsychotic treatment (maximum 4 weeks) for FEPs. Exclusion criteria were the presence of mental retardation, neurological or organic mental disorders, and substance abuse history other than nicotine. The FEPs were followed for approximately 1 year to confirm the diagnosis. One patient who was diagnosed with the first episode was later diagnosed with neurological (Fahr) disease and excluded. Three patients were excluded because of bipolar mood disorder with psychotic features, and one patient was excluded because the MRS spectra values were inappropriate. Consequently, the FEP consisted of 19 patients. The control group was composed of 30 healthy volunteer subjects who were age- and sex-matched, between 18 and 60 years of age, and working at the hospital. Axis I diagnoses were evaluated by using a semistructured psychiatric form (SCID-I) (32). For the control group, exclusion criteria were defined as fulfillment of diagnostic criteria for any psychiatric disorder, presence of neurological or organic mental disorder, and mental retardation. Patients and their relatives were informed about the objective and procedures of the study, and written informed consent was obtained either from the patient or their relatives. After obtaining the informed consent, patients and control subjects were included in the study. The research project was approved by the Local Ethics Committee of the Medical School of Pamukkale University, and the study was supported by the Scientific Research Projects Commission of the Medical Faculty of Pamukkale University (Project Number 2011TPF004).

Severity of symptoms was evaluated in the patient group by using the Scale for Assessment of Negative Symptoms (SANS) (33), Scale for Assessment of Positive Symptoms (SAPS) (34), and Clinic Global Impression Severity Scale (CGI) (35).

2.1. Medication status

Among the FEPs, 18 were receiving atypical antipsychotic agents (12 olanzapine, 4 risperidone, 2 aripiprazole) and 1 patient was drug-free.

Among the CSPs, 15 patients were receiving atypical antipsychotics (5 aripiprazole, 3 olanzapine, 3 paliperidone, 2 risperidone, 2 ziprasidone), 9 patients were receiving dual atypical antipsychotics, 1 patient was receiving a typical antipsychotic (flupentixol), and 5 patients were receiving atypical and typical depot antipsychotic combinations. In the CSP group, 2 patients were receiving sodium valproate in addition to their ongoing antipsychotic treatment.

2.2. Proton magnetic resonance spectroscopy procedure

Proton MRS (^{1}H-MRS) examinations were performed using a 1.5-T magnetic resonance device (GE Medical System, Milwaukee, WI, USA) with a standard head coil. First, a complete brain guiding image was taken at the sagittal plane to define consecutive sequence positions and for orientation. Sequentially, the magnetic resonance protocol was completed in the coronal plane at 10 mm of thickness by taking the T2 weighted fast spin echo sequence using time of repetition/time of echo (TR/TE): 3000/85, field of view: 14, matrix: 352 × 352, and number of excitations: 1. MRS was performed by a single voxel (^{1}H-voxel) technique implemented in the right prefrontal cortex, ACC, left thalamus, and left hippocampus areas (Figure 1). To define the voxel position and to minimize differences of implementation between subjects, a standard approach was used by using identifiable anatomical landmarks on a reference human brain atlas (36). The examined volume amount (volume of interest, VOI) was defined as 20 × 20 × 20 mm^3 for each voxel, and it was implemented so that the related brain tissue in the frontal lobe was definitely covered. The chemical shift selective pulse (37) method was used to suppress water origin signals. Consequently, the point-resolved spectroscopy technique (38), which localizes spectroscopy volume, was used (TR/TE: 3000/35). Finally, short-time TE spectrums were obtained from the VOIs in the examined areas. For the quality control of spectroscopy, spectroscopy was regularly performed on the spectroscopy phantom, which was present in the device and had all metabolites, and quality control of the device was performed by observing the obtained spectrum quality. Additionally, standard signal-to-noise ratios (defined as the noise standard deviation ratio of NAA value), which were obtained from the obtained patient resonances and the software program existing in the device, were recorded, and spectra values below 3 were excluded from the measurements. Moreover, studies at 6 Hz, which was the full width at half maximum of water spectrum, measured during sending the spectroscopy sequence by the device or lower than 6 Hz were included in the measurements. Obtained data were evaluated by the General Electric software spectral analysis program (Figure 2) Investigating levels of NAA-, Cho-, and Cre-containing compounds in the DLPFC, ACC, thalamus, and hippocampus areas, NAA/Cre and Cho/Cre levels were calculated. The results were analyzed by a radiology specialist.

2.3. Statistical analysis

Statistical analysis was performed using SPSS 15.0. Intergroup differences in categorical variables were analyzed by chi-square test. Intergroup differences in continuous variables were analyzed by one-way analysis of variance (age, education level) when comparing three groups, and differences in two groups (i.e. clinical rating scores of FEPs and CSPs) were analyzed by Student t-test. In order to investigate whether MRS metabolite values and metabolite ratios differed between the groups we used multivariate analysis of covariance (MANCOVA) analysis. In these analyses neurometabolite levels or ratios for each examined area were defined as dependent variables, groups as between-subject factors, and age and sex as covariate**.** We used the Bonferroni test for post hoc pairwise comparisons. Correlations between disease

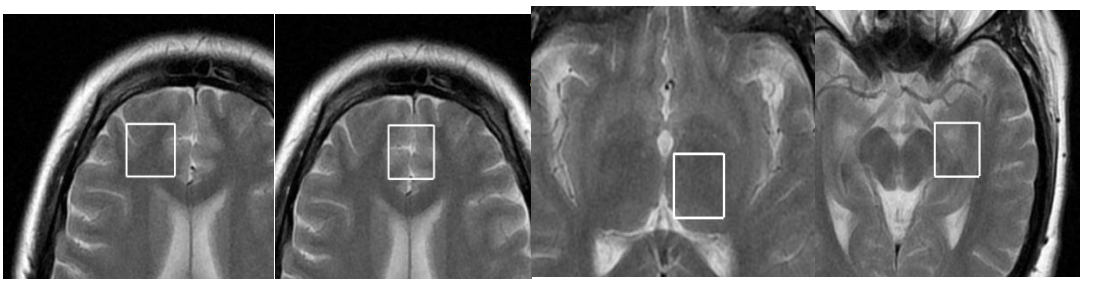

Figure 1. H^1-MRS application implemented in the DLPFC, ACC, thalamus, and hippocampus areas by using a single voxel technique.

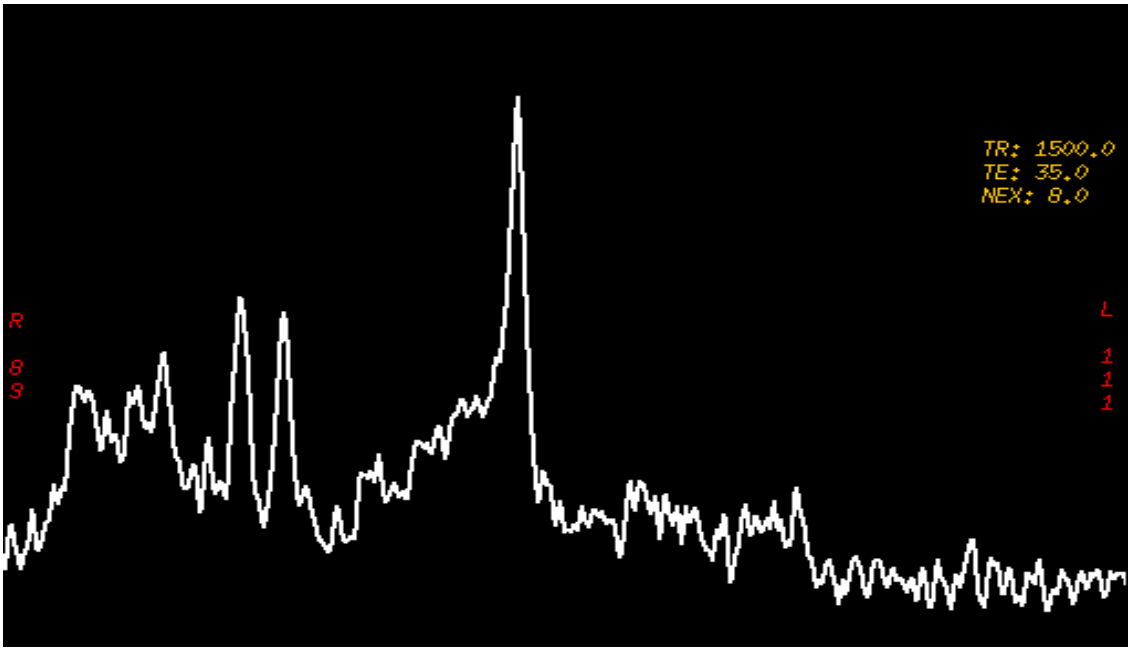

Figure 2. Graphic presentation of metabolite levels and the obtained peak values in the areas by the H^1-MRS study.

onset age, disease durations and clinical scale points, and metabolite levels or ratios showing intergroup differences were examined by performing Pearson correlation analysis.

3. Results

There was no statistically significant difference in age, sex, or education level between the groups. For clinical scales, there was no statistically significant difference in SANS and CGI scores between the groups, but SAPS scores were significantly higher in the FEPs when compared with the CSPs (Table 1). The mean duration of the illness was 69.94 ± 90.18 days for FEPs and 10.60 ± 6.99 years for CSPs. There was no statistically significant difference between the mean ages of female (23.42 ± 7.86) and male (24.11 ± 11.22) CSPs, while the mean age of females (33.90 ± 10.65) was significantly higher than that of male patients (24.22 ± 6.18) among FEPs (P = 0.029).

3.1. DLPFC metabolite levels and metabolite ratios

In MANCOVA analysis, a significant difference was determined in neurometabolites and neurometabolite ratios in the DLPFC between the groups (respectively Wilks's lambda = 0.672, F = 2.588, P = 0.013; Wilks's lambda = 0.616, F = 3.285, P = 0.002). There were significant differences in DLPFC Cho level and DLPFC NAA/Cho ratio between the groups (Table 2). DLPFC Cho levels were higher in CSPs than healthy controls (95% CI for difference, lower bound = 1.177; upper bound = 12.752, P = 0.011). The NAA/Cho ratio was lower in both the FEPs and CSPs than the healthy controls (respectively 95% CI for difference, lower bound = –0.558, upper bound = –0.193, P = 0.000 and lower bound = –0.472, upper bound = –0.056, P = 0.008). There was a decreasing but insignificant trend in the NAA/Cre ratio in CSP when compared with the controls, whereas there was an increasing trend in Cho/Cre ratio (P = 0.078 and P = 0.079, respectively). There was no significant effect of age or sex on DLPFC metabolite levels or ratios.

3.2. Hippocampus metabolite levels and metabolite ratios

Hippocampal ^{1}H-MRS metabolite levels were obtained in 14 FEPs, 22 CSPs, and 26 controls. Hippocampal NAA levels were significantly different between the groups (Wilks's lambda = 0.793, F = 3.138, P = 0.018). In post hoc pairwise analysis, it was determined that NAA levels in CSPs were significantly lower than in the control group (P = 0.003) (95% CI lower bound = –18.689, upper bound = –4.915). Hippocampal metabolite levels or ratios in the FEPs were not different from the control group. There was no significant age or sex effect on hippocampus metabolite levels or ratios. There was no significant difference in neurometabolite levels and ratios in the ACC and thalamus between the groups.

3.3. Correlations between neurometabolite levels and clinical variables

In the FEP group DLPFC NAA/Cho ratio and SANS scores were negatively correlated (r = –0.462, P = 0.050). In the CSP group, SANS scores were positively correlated with DLPFC Cho levels (r = 0.376, P = 0.044) and negatively correlated with DLPFC NAA/Cho ratio (r = –0.455, P = 0.013). Additionally, there was a significant negative

Table 1. Sociodemographic and clinical characteristics of diagnostic groups.

	First episode psychosis		Chronic schizophrenia		Control			
	Number	%	Number	%	Number	%	Test statistics	P
Sex								
Female	10	52.6	12	40.0	14	46.7	$c^2 = 0.772$	0.68
Male	9	47.4	18	60.0	16	53.3		
	Mean	SD	Mean	SD	Mean	SD		P
Age	29.32	9,92	34.33	11.19	30.93	6.58	F = 1.897	0.157
Education duration	10.50	3.63	8.56	3.19	11.23	3.37	F = 4.75	0.073
SANS	31.63	20.36	27.13	14.21			t = 0.911	0.367
SAPS	29.11	15.14	19.33	9.65			t = 2.508	0.018
CGI	4.84	0.95	4.46	0.72			t = 1.687	0.073

correlation between NAA/Cho ratio and age at onset in CSPs (r = –0.416, P = 0.025).

4. Discussion

In the present study, brain metabolite levels of FEPs and CSPs were compared with those of healthy controls. The main findings were lower NAA/Cho ratios and higher Cho levels in the DLPFC and lower hippocampal NAA levels in the CSPs compared to the healthy controls. In the FEP group, the DLPFC NAA/Cho ratio was lower than in the control group. The DLPFC NAA/Cho ratio showed a significant correlation with negative symptom severity in both the FEPs and CSPs.

In studies with participants similar to ours, such as those with approximately equal female/male ratio and patients receiving short-term antipsychotic treatment, it was reported that NAA/Cre ratios in the prefrontal cortex, thalamus, temporal lobe, and hippocampus in FEPs were not different from those of healthy individuals (17,39–42). On the other hand, similar to our results, Bertolino et al. reported that NAA/Cho and NAA/Cre ratios were decreased in the DLPFC and hippocampus in patients with schizophreniform disorder (13). Jessen et al. reported that NAA/Cho ratio was decreased in the frontal lobe in FEPs, and low NAA/Cho ratio and high Cho/Cre ratio in the ACC were related to switching to chronic schizophrenia (43). It was also reported that CSPs had lower NAA/Cho levels than healthy individuals, and it was proposed that the decrease in NAA/Cho ratio reflected the presence of neuronal loss (9,44).

The Cho peak is a complex formed by mainly phosphoryl choline and glycerophosphoryl choline. The Cho peak is elevated in tumors and inflammatory or neurodegenerative processes (45,46). It was reported that Cho levels in the frontal lobe were not generally changed or increased in CSPs (29,30,47). High Cho concentrations may reflect decreased glucose metabolism or abnormalities in the phospholipid membrane formation (30,31,47–49).

The issue that must be discussed here is that decrease in DLPFC NAA/Cho levels may be a secondary condition emerging in association with Cho increase in this region. Although Cho levels in the FEPs were not different from healthy individuals, decrease in NAA/Cho ratio and similarity of DLPFC Cho/Cre ratios between patient and control groups indicate that this condition may not be a secondary sign only, but it may be related to the possible change in the NAA level. Some studies that compared CSPs and FEPs with healthy individuals reported that there were significant decreases in DLPFC NAA or NAA/Cre ratios in both patient groups (7,8,27). In the present study, no significant difference was observed in DLPFC NAA or NAA/Cre ratios between CSPs and the healthy control group. Follow-up studies have reported that NAA levels were not low at the baseline; the decrease occurred within the first year after the symptoms had started (7,10,50). The onset of symptoms among our FEPs was approximately 70 days; this might cause the lack of difference in NAA levels between the patients and healthy individuals.

Moreover, atypical antipsychotic treatment may have acted as a confounding factor for DLPFC NAA levels. Cross-sectional and longitudinal studies have proposed that atypical antipsychotics normalized the decreased NAA levels (10,13,24). It has been reported that NAA levels selectively increased in the DLPFC within 4 weeks but not in the temporal lobe (13). In our study, the FEPs included patients receiving minimal antipsychotic treatment (up to 4 weeks), whereas CSPs were being treated mainly by atypical antipsychotics. This condition might prevent us from determining NAA changes.

With significant decrease in the DLPFC NAA/Cho ratio in both patient groups compared to healthy controls

Table 2. Metabolite levels and metabolite ratios in the DLPFC and hippocampus.

	First episode (n = 19)		Chronic sch (n = 30)		Controls (n = 30)		Post hoc comparisons	
DLPFC	Mean	SD	Mean	SD	Mean	SD	F	P
NAA	60.36	12.55	61.10	12.2	62.40	10.20	0.462	0.561
Cre	38.55	6.61	39.33	8.17	36.96	5.93	0.946	0.382
Cho	39.26	9.15	40.39	8.91	34.81	5.82	4.956	0.011 CSP > C
Myo-I	29.07	5.02	29.30	5.24	27.55	4.92	0.645	0.528
NAA/Cre	1.61	0.25	1.58	0.30	1.72	0.23	2.689	0.078
NAA/Cho	1.60	0.21	1.57	0.27	1.84	0.28	13.618	0.000 CSP, FE < C
Cho/Cre	1.02	0.19	1.03	0.20	0.94	0.14	2.675	0.079
Myo/Cre	0.71	0.11	0.75	0.20	0.72	0.16	0.164	0.849
Hippocampus	(n = 14)		(n = 22)		(n = 26)			
NAA	54.64	14.29	45.73	11.06	56.85	12.09	6.612	0.003 CSP < C
Cre	37.21	9.69	32.86	6.32	38.38	7.07	2.262	1.113
Cho	37.50	10.79	31.04	7.42	37.86	8.39	3.077	0.055
Myo-I	29.91	6.06	28.56	3.16	28.65	3.77	1.090	0.342
NAA/Cre	1.53	0.25	1.42	0.18	1.50	0.16	1.956	0.149
NAA/Cho	1.56	0.37	1.44	0.26	1.60	0.27	0.276	0.760
Cho/Cre	1.03	0.23	0.97	0.17	0.95	0.17	0.288	0.751
Myo-I/Cre	0.84	0.13	0.88	0.20	0.75	0.16	0.590	0.559

and significant increase in DLPFC Cho levels in chronic patients, DLPFC neuronal integrity might be impaired at the onset of schizophrenia. NAA changes might not be detected during treatment, but impaired neuronal density or disturbed energy metabolism might persist starting from the first episode to the chronic stages. In the present study, the DLPFC NAA/Cho ratio was significantly correlated with negative symptom severity in both FEPs and CSPs. We also found that DLPFC Cho levels in chronic patients were significantly correlated with negative symptoms and age at onset. In previous studies, a negative correlation was also reported between PFC NAA/Cre ratio and negative symptoms, which supports the association of the frontal cortex with negative symptoms in schizophrenia (12,51).

Starting with Nasrallah et al.'s study, a decreased hippocampal NAA level was supported by several other studies in schizophrenia patients, and it was reported that NAA decrease in the hippocampus was approximately 22% (52). Considering that the decrease in hippocampal NAA levels is a more consistent result when compared to those in other brain areas such as the frontal cortex, basal ganglion, thalamus, and cingulate cortex in CSPs, it may be interpreted as strong evidence for neuronal/axonal loss in the hippocampus in schizophrenia (14,16,20,53–55). In our study, similar to these results, NAA levels in the hippocampus were significantly decreased in the CSPs when compared to healthy controls. Consistent with previous reports, this finding was not found in the FEP group, so it suggests a progressive process in schizophrenia (14–16,48).

It was observed that there was no difference in ACC metabolite levels between CSPs, FEPs, and healthy controls in the majority of studies (20–22,25). The common characteristic of studies reporting decreases in ACC NAA levels is that they were performed on chronic patients under typical antipsychotic treatment (23–25). In a recent and comprehensive review, it was reported that when looking at the methodologically rigorous studies, no NAA, Cho, Cre, or myo-I alterations could be found in schizophrenia patients (49,56). Consistent with the literature, there was no difference in NAA levels in the ACC in CSPs and FEPs when compared with the healthy individuals in the present study.

It was reported that there was neuronal loss, decreased volume, and synaptic degeneration in the thalamus of schizophrenia patients consistently with thalamic dysfunction in schizophrenia pathogenesis (57–60). When MRS studies on FEPs were evaluated, similar to our results, NAA, NAA/Cho, Cho/Cre, and NAA/Cre values in the thalamus were not different from those of healthy controls

in schizophreniform cases or FEPs (18,61). It was also reported that Cho, Cre, and myo-I levels in the thalamus were not different in the FEPs from healthy controls (39).

In the present study, thalamic metabolites in CSPs were not different from those of healthy controls. Results of studies examining thalamic metabolite levels in CSPs are divergent. While there was no significant difference in metabolite levels and ratios when compared with the control cases (20,21,62–64), decreases in NAA and NAA/Cre ratios were reported in some studies. It was determined that thalamic NAA, Cre, and Cho levels were decreased in male patients, children and adolescent patients with schizophrenia, and individuals who had high genetic risk for schizophrenia (4,28,65). Additionally, it has been reported that decreased NAA or NAA/Cho levels were associated with acute psychotic attack, active auditory hallucinations, and typical antipsychotic use in the CSP group (66–69). Considering these findings, our patient characteristics and atypical antipsychotic treatment might have affected our results.

When the limitations of our study are considered, our sample size is quite similar to the previous H^1-MRS studies. However, a metaanalysis evaluating MRS findings considered that 39 patients and 39 controls are required for acceptable statistical power, and so our sample size may be accepted as relatively limited (3,70). The present study was performed by using a 1.5-T MRS device with a short TE. The single voxel MRS procedure used in the present study is methodologically comparable with many previous studies performed with this field strength. However, it was reported that MRS techniques with higher magnetic field strength and high resolution power would facilitate signal perception and thus increase the sensitivity (49,56). In addition, we did not evaluate glutamate and glutamine levels, which might be differentiated in spectra using MRS protocols with higher magnetic field strength. As previously reported, not excluding the drug effects on brain metabolites is another limitation of our study (13,71–74).

The advantages of our study are that the sexes were represented at a nearly equal ratio among both the FEPs and CSPs, the diagnoses of FEPs were confirmed by follow-up, patient groups were composed of age-matched individuals, and age and sex effects on MRS metabolites were controlled in the statistical analyses. Moreover, we included absolute metabolite values or ratios of the investigated brain area in MANCOVA and analyzed the intergroup metabolite differences by using a multivariable covariant analysis to decrease the possible type II error. Therefore, we think that our results are reliable for controlling the confounding factors in statistical analysis. The present study is also one of studies comparing FEPs and CSPs with healthy controls in the same study design and evaluating the DLPFC, ACC, thalamus, and hippocampus, which are thought to be involved in schizophrenia etiology at the same time.

In conclusion, our results showing higher Cho levels and lower NAA/Cho ratios in the DLPFC as well as lower NAA levels in the hippocampus in CSPs when compared with the healthy controls are consistent with the impaired neuronal integrity and membrane phospholipids reported in the advanced stages of the disease by previous studies (19). Decreased NAA/Cho ratio in FEPs when compared to the healthy controls supports the role of DLPFC dysfunction in schizophrenia development and also suggests existent neuronal dysfunction starting at the onset of disease. Further longitudinal studies with larger sample sizes with FEPs followed for longer periods of time, with better control on the drug effects, and with higher resolutions of MRS technique are required.

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